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#6	Search **protease and bacillus and alkaline and chromosom***Limits: **Publication Date to 1989/08/11**	08:40:06	9
#5	Search **protease and bacillus and alkaline and chromosom$**Limits: **Publication Date to 1989/08/11**	08:39:58	0
#3	Search **protease and bacillus and alkaline**Limits: **Publication Date to 1989/08/11**	08:39:07	100
#2	Search **protease and bacillus** Limits: **Publication Date to 1989/08/11**	08:37:04	997
#1	Search **protease and bacillus**	08:36:49	2097

Clear History

☐ 1: *J Biochem (Tokyo)* 1988 Sep;104(3):416-20

Related Articles, Books

Specificity of alkaline elastase Bacillus on the oxidized insulin A- and B-chains.

Tsai YC, Lin YT, Yang YB, Li YF, Yamasaki M, Tamura G

Institute of Biochemistry, National Yang-Ming Medical College, Taipei, Taiwan.

The substrate specificity of alkaline elastase Bacillus from alkalophilic Bacillus sp. Ya-B was investigated using oxidized insulin A- and B-chains. Under time-limited cleavage, the initial cleavage site of the enzyme on the oxidized insulin A-chain and B-chain was at the leucine13-tyrosine14 bond and the leucine15-tyrosine16 bond, respectively. When the cleavage was completed, three major cleavage sites and three minor cleavage sites on the A-chain, and five major cleavage sites and four minor cleavage sites on the B-chain were found. However, most of the peptides produced after complete hydrolysis of the A- or B-chain by the enzyme were composed of four to six amino acid residues. The results suggest that this enzyme cleaves the oxidized insulin A- and B-chains in a block-cutting manner.

MeSH Terms:

- Amino Acids/analysis
- Bacillus/enzymology*
- Chromatography, High Pressure Liquid
- Hydrolysis
- Insulin/metabolism*
- Oxidation-Reduction
- Pancreatic Elastase/metabolism*
- Substrate Specificity
- Support, Non-U.S. Gov't

Substances:

- Pancreatic Elastase
- alkaline elastase
- Insulin
- Amino Acids

PMID: 3071529

☐ 2: *J Bacteriol* 1987 Jun;169(6):2762-8 Related Articles, Books

Novel alkaline- and heat-stable serine proteases from alkalophilic Bacillus sp. strain GX6638.

Durham DR, Stewart DB, Stellwag EJ

An alkalophilic Bacillus sp., strain GX6638 (ATCC 53278), was isolated from soil and shown to produce a minimum of three alkaline proteases. The proteases were purified by ion-exchange chromatography and were distinguishable by their isoelectric point, molecular weight, and electrophoretic mobility. Two of the proteases, AS and HS, which exhibited the greatest alkaline and thermal stability, were characterized further. Protease HS had an apparent molecular weight of 36,000 and an isoelectric point of approximately 4.2, whereas protease AS had a molecular weight of 27,500 and an isoelectric point of 5.2. Both enzymes had optimal proteolytic activities over a broad pH range (pH 8 to 12) and exhibited temperature optima of 65 degrees C. Proteases HS and AS were further distinguished by their proteolytic activities, esterolytic activities, sensitivity to inhibitors, and their alkaline and thermal stability properties. Protease AS was extremely alkali stable, retaining 88% of initial activity at pH 12 over a 24-h incubation period at 25 degrees C; protease HS exhibited similar alkaline stability properties to pH 11. In addition, protease HS had exceptional thermal stability properties. At pH 9.5 (0.1 M CAPS buffer, 5 mM EDTA), the enzyme had a half-life of more than 200 min at 50 degrees C and 25 min at 60 degrees C. At pH above 9.5, protease HS readily lost enzymatic activity even in the presence of exogenously supplied Ca2+. In contrast, protease AS was more stable at pH above 9.5, and Ca2+ addition extended the half-life of the enzyme 10-fold at 60 degrees C. (ABSTRACT TRUNCATED AT 250 WORDS)

MeSH Terms:

- Bacillus/metabolism
- Bacillus/enzymology*
- Endopeptidases/metabolism*
- Endopeptidases/isolation & purification
- Endopeptidases/immunology
- Fermentation
- Heat
- Hydrogen-Ion Concentration
- Immunodiffusion
- Molecular Weight
- Protease Inhibitors
- Serine Endopeptidases

Substances:

- Serine Endopeptidases
- Endopeptidases
- Protease Inhibitors

PMID: 3108241

☐ 3: *Biochem Int* 1984 Feb;8(2):283-8 Related Articles, Books

Substrate specificity of a new alkaline elastase from an alkalophilic bacillus.

Tsai YC, Yamasaki M, Tamura G

The substrate specificity of alkaline elastase from alkalophilic Bacillus sp. Ya-B was studied by using a number of synthetic substrates. From the relative hydrolysis rate for p-nitrophenyl esters and t-butoxycarbonyl-L-Phe-L-Arg(NO2)-X-L-Phe-p-nitroanilide (X = L-Ala, Val, Leu, Ile, and Gly), the subsite S1 and S2 were concluded to be specific for L-alanine and glycine. The alkaline elastase rapidly hydrolyzed elastase specific substrate succinyl-L-Ala3-p-nitroanilide and succinyl-L-Ala-L-Pro-L-Ala-p-nitroanilide. These results prompted us to characterize our enzyme as a microbial elastase. Inhibition study with carbobenzoxy-L-Phe-chloromethyl ketone (ZPCK), Z-L-Ala-L-Phe-CK (ZAPCK), Z-L-Ala-Gly-L-Phe-CK (ZAGPCK), and kinetic study with succimyl-L-Ala2(3)-p-nitroanilide revealed that the enzyme has at least four subsites.

MeSH Terms:
- Bacillus/enzymology*
- Hydrogen-Ion Concentration
- Kinetics
- Pancreatic Elastase/metabolism*
- Protease Inhibitors/pharmacology
- Substrate Specificity
- Support, Non-U.S. Gov't

Substances:
- Pancreatic Elastase
- alkaline elastase
- Protease Inhibitors

PMID: 6566572

☐ 4: *Biochem Int* 1983 Nov;7(5):577-83 Related Articles, Books

A new alkaline elastase of an alkalophilic bacillus.

Tsai YC, Yamasaki M, Yamamoto-Suzuki Y, Tamura G

A new alkaline elastase was purified from the culture broth of an alkalophilic Bacillus sp. Ya-B. This was a serine proteinase. Molecular weight was 25,000. The optimum pH for elastin and casein was 11.75. The enzyme had very high specific activity, 12,400 units/mg protein for casein, and 2,440 units/mg protein for elastin at the optimum pH. It showed marked preference for elastin. The relative activity of elastin/casein of this enzyme was 17 and 6 times higher than those of subtilisin BPN' and subtilisin Carlsberg, respectively. This enzyme also had higher keratin and collagen hydrolyzing activity in comparison with subtilisin.

MeSH Terms:
- Bacillus/enzymology*
- Comparative Study
- Endopeptidases/metabolism
- Endopeptidases/isolation & purification*
- Kinetics

- Molecular Weight
- Pancreatic Elastase/metabolism
- Pancreatic Elastase/isolation & purification*
- Protease Inhibitors/pharmacology
- Serine Endopeptidases
- Substrate Specificity
- Support, Non-U.S. Gov't

Substances:

- Pancreatic Elastase
- alkaline elastase
- Serine Endopeptidases
- Endopeptidases
- Protease Inhibitors

PMID: 6385982

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=> d his

     (FILE 'HOME' ENTERED AT 15:35:55 ON 28 MAR 2001)

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     BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CANCERLIT,
     CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE,
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MAR
     2001
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                3   FILE BIOBUSINESS
               10   FILE BIOSIS
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                8   FILE WPIDS
                8   FILE WPINDEX
L1              QUE PB92
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     FILE 'TOXLINE, DGENE, NTIS, USPATFULL, AQUASCI, AGRICOLA, CAPLUS,
IFIPAT,
     SCISEARCH, BIOTECHDS, LIFESCI, BIOSIS, MEDLINE, CEABA-VTB, WPIDS,
     GENBANK, EMBASE, PROMT, BIOTECHNO, FSTA, PASCAL, TOXLIT, BIOBUSINESS,
     CANCERLIT, ESBIOBASE, CEN, OCEAN' ENTERED AT 15:42:14 ON 28 MAR 2001
L2          1975 S L1
L3           331 S L2 AND MUT?
L4           292 S L3 AND BACILLUS?
L5           263 DUP REM L4 (29 DUPLICATES REMOVED)
L6           268 S L4 AND ALKAL?
L7             6 S L6 AND PY<=1990
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=> d ibib ab 1

L7 ANSWER 1 OF 6 USPATFULL
ACCESSION NUMBER: 94:68700 USPATFULL
TITLE: **PB92** serine protease **muteins** and their use in detergents
INVENTOR(S): van Eekelen, Christiaan A. G., Bergschenhoek, Netherlands
Mulleners, Leonardus J. S. M., SV Rijen, Netherlands
Van Der Laan, Johannes C., Amsterdam, Netherlands
Misset, Onno, Delft, Netherlands
Cuperus, Roelck A., Amsterdam, Netherlands
Lensink, Johan H. A., Delft, Netherlands
PATENT ASSIGNEE(S): Gist-brocades N.V., Delft, Netherlands (non-U.S. corporation)

	NUMBER	DATE	
PATENT INFORMATION:	US 5336611	19940809	
	WO 8907642	19890824	
APPLICATION INFO.:	US 1989-427103	19891011	(7)
	WO 1989-NL5	19890213	
		19891011	PCT 371 date
		19891011	PCT 102(e) date

	NUMBER	DATE
PRIORITY INFORMATION:	EP 1988-200255	19880211

DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Robinson, Douglas W.
ASSISTANT EXAMINER: Weber, Jon P.
LEGAL REPRESENTATIVE: Rae-Venter, Barbara
NUMBER OF CLAIMS: 8
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 8 Drawing Figure(s); 8 Drawing Page(s)
LINE COUNT: 1339
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB New proteolytic enzymes are provided exhibiting improved properties for application in detergents, especially laundry detergents. These enzymes are obtained by expression of a gene encoding a proteolytic enzyme having an amino acid sequence which differs at least in one amino acid from the wild type enzyme. Preferred enzymes are certain **mutants** derived from the serine protease of **Bacillus** nov. spec. **PB92**.

=> d ibib ab 2

L7 ANSWER 2 OF 6 USPATFULL
ACCESSION NUMBER: 88:59037 USPATFULL
TITLE: Heat stable **alkaline** proteases produced by a **bacillus**
INVENTOR(S): Stellwag, Edmund J., Damascus, MD, United States
Durham, Donald R., Gaithersburg, MD, United States
Swann, Wayne E., Columbia, MD, United States
Nolf, Carol A., Silver Spring, MD, United States
Stewart, David B., Arlington, VA, United States
PATENT ASSIGNEE(S): Genex Corporation, Gaithersburg, MD, United States

(U.S. corporation)

	NUMBER	DATE	
PATENT INFORMATION:	US 4771003	19880913	<--
APPLICATION INFO.:	US 1985-790256	19851022 (6)	

DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Shapiro, Lionel M.
LEGAL REPRESENTATIVE: Saidman, Sterne, Kessler & Goldstein
NUMBER OF CLAIMS: 14
EXEMPLARY CLAIM: 1
LINE COUNT: 637
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Novel enzymes exhibiting proteolytic activity in **alkaline** media and stability at high temperatures and under **alkaline** conditions are produced by a novel **Bacillus** strain designated GX6638 or its **mutants** or variants. These enzymes are especially well-suited for inclusion in washing compositions. A culture of GX6638 has been deposited with the American Type Culture Collection, Rockville, Md. as ATCC No. 53278.

=> d ibib ab 3

L7 ANSWER 3 OF 6 USPATFULL
ACCESSION NUMBER: 88:52046 USPATFULL
TITLE: **Alkaline** protease produced by a **bacillus**
INVENTOR(S): Durham, Donald R., Gaithersburg, MD, United States
Stellwag, Edmund J., Greenville, MD, United States
McNamee, Clyde G., Gaithersburg, MD, United States
PATENT ASSIGNEE(S): Genex Corporation, Gaithersburg, MD, United States (U.S. corporation)

	NUMBER	DATE	
PATENT INFORMATION:	US 4764470	19880816	<--
APPLICATION INFO.:	US 1986-826378	19860205 (6)	

DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Shapiro, Lionel M.
LEGAL REPRESENTATIVE: Saidman, Sterne, Kessler & Goldstein
NUMBER OF CLAIMS: 11
EXEMPLARY CLAIM: 1
LINE COUNT: 479
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A novel enzyme exhibiting proteolytic activity in **alkaline** media and stability under **alkaline** conditions is produced by a novel **Bacillus** strain designated GX6644 or by its **mutants** or variants. The enzyme is well-suited for inclusion in washing compositions. A culture of GX6644 has been deposited with the American Type Culture Collection, Rockville, Maryland as ATCC No. 53441.

=> d ibib ab 4

L7 ANSWER 4 OF 6 USPATFULL
ACCESSION NUMBER: 85:22293 USPATFULL
TITLE: Cooperative enzymes comprising **alkaline** or mixtures of **alkaline** and neutral proteases without stabilizers
INVENTOR(S): Stanislowski, Anna G., Tracy, CA, United States
Wiersema, Richard J., Pleasanton, CA, United States
PATENT ASSIGNEE(S): The Clorox Company, Oakland, CA, United States (U.S.

corporation)

	NUMBER	DATE	
PATENT INFORMATION:	US 4511490	19850416	<--
APPLICATION INFO.:	US 1983-508449	19830627 (6)	

DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Kittle, John E.
ASSISTANT EXAMINER: Shah, Mukund J.
LEGAL REPRESENTATIVE: Hayashida, Joel J.; Westbrook, Stephen M.
NUMBER OF CLAIMS: 33
EXEMPLARY CLAIM: 1
LINE COUNT: 846
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A stable, cooperative enzyme system which is stable under use conditions is disclosed which comprises at least two enzymes having activity towards a relatively complex substrate with at least partial activity over the same pH range, wherein their combined activities are greater than the sum of their individual activities as determined by the formula: ##EQU1## wherein E.sub.1 and E.sub.2 are said enzymes. No additional chemical stabilizers, modifiers or activators are added to the enzymes of this invention.

Particularly preferred enzymes in this invention are proteases having optimal activity in acidic, neutral or **alkaline** media, and mixtures of the same.

A method of making this cooperative enzyme system is also disclosed.

The enzyme systems of this invention have a wide variety of uses in cleaning and other applications.

=> d ibib ab 5

L7 ANSWER 5 OF 6 BIOTECHDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1988-12809 BIOTECHDS
TITLE: Cloning and expression of genes encoding proteolytic enzyme; e.g. serine protease gene expression in **Bacillus** sp.
PATENT ASSIGNEE: Brocades
PATENT INFO: WO 8806624 **7 Sep 1988**
APPLICATION INFO: WO 1988-N
L7 26 Feb 1988
PRIORITY INFO: NL 1987-200358 27 Feb 1987
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1988-271164 [38]

AB A new expression cassette comprises transcriptional regulatory, transcriptional initiation and translational initiation regions (which are functional in **Bacillus** sp. host), DNA encoding an **alkaline** protease, and transcriptional and translational termination regions. Preferably at least 1 marker gene and a temp.-sensitive bacterial origin of replication are present. The enzyme gene is preferably from **Bacillus** sp. strain **PB92** or has at least 90% homology with it. Also new are: (1) a transformed wild-type (or **mutant**), serine protease (non-) producing **Bacillus** sp. host, preferably containing at least 2 copies of the enzyme DNA sequence in its chromosome; (2) a method for producing serine protease in an **alkalophilic Bacillus** sp. host which comprises growing the transformed host in a culture medium under conditions which promote enzyme over-production; (3) a method for transforming **alkalophilic Bacillus** cells which comprises pretreating **Bacillus** cells with lysozyme

(EC-3.2.1.17) at 20-37 deg to form protoplasts and introducing DNA in the presence of a fusogen; (4) plasmid pMAX-4; and (5) DNA encoding the serine protease. (32pp)

=> d ibib ab 6

L7 ANSWER 6 OF 6 BIOTECHDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1988-12322 BIOTECHDS
TITLE: Transformed prokaryotic host cell; alpha-amylase or serine protease gene amplification in **Bacillus** licheniformis or **Bacillus** sp.
PATENT ASSIGNEE: Brocades
PATENT INFO: WO 8806623 **7 Sep 1988**
APPLICATION INFO: WO 1988-N L6 26 Feb 1988
PRIORITY INFO: NL 1987-200356 27 Feb 1987
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1988-271163 [38]

AB A transformed prokaryotic host cell comprises at least 2 copies of a DNA sequence in its chromosome. The DNA sequence amplified encodes a desired polypeptide and the copies are separated by endogenous chromosomal DNA sequences. The prokaryotic cell is produced by combining a recipient host cell (comprising at least 1 copy of the desired DNA sequence) with a DNA construct, preferably plasmid pMAX-4 or pE1atB, which contains either (a) at least 1 copy of the DNA sequence and at least 1 marker gene and a temp.-sensitive replication origin or (b) a donor host cell comprising the DNA construct, under transforming conditions. The transformed prokaryote is selected, e.g. on account of its resistance to a pesticide or antibiotic and isolated. The transformed prokaryote is preferably a **Bacillus** sp., especially **Bacillus** licheniformis (e.g. strain T5), an **alkalophilic Bacillus** strain novo sp. **PB92**, **Bacillus** strains PBT108, pBT122, or T13F or their **mutants** or variants. The desired polypeptide amplified is preferably a proteolytic, or an amylolytic enzyme, especially a serine protease or alpha-amylase (EC-3.2.1.1). (59pp)

L7 ANSWER 5 OF 6 BIOTECHDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1988-12809 BIOTECHDS
TITLE: Cloning and expression of genes encoding proteolytic enzyme; e.g. serine protease gene expression in **Bacillus** sp.
PATENT ASSIGNEE: Brocades
PATENT INFO: WO 8806624 **7 Sep 1988**
APPLICATION INFO: WO 1988-N
L7 26 Feb 1988
PRIORITY INFO: NL 1987-200358 27 Feb 1987
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1988-271164 [38]
AB A new expression cassette comprises transcriptional regulatory, transcriptional initiation and translational initiation regions (which are functional in **Bacillus** sp. host), DNA encoding an **alkaline** protease, and transcriptional and translational termination regions. Preferably at least 1 marker gene and a temp.-sensitive bacterial origin of replication are present. The enzyme gene is preferably from **Bacillus** sp. strain **PB92** or has at least 90% homology with it. Also new are: (1) a transformed wild-type (or **mutant**), serine protease (non-) producing **Bacillus** sp. host, preferably containing at least 2 copies of the enzyme DNA sequence in its chromosome; (2) a method for producing serine protease in an **alkalophilic Bacillus** sp. host which comprises growing the transformed host in a culture medium under conditions which promote enzyme over-production; (3) a method for transforming **alkalophilic Bacillus** cells which comprises pretreating **Bacillus** cells with lysozyme (EC-3.2.1.17) at 20-37 deg to form protoplasts and introducing DNA in the presence of a fusogen; (4) plasmid pMAX-4; and (5) DNA encoding the serine protease. (32pp)
TI Cloning and expression of genes encoding proteolytic enzyme; e.g. serine protease gene expression in **Bacillus** sp.
PI WO 8806624 **7 Sep 1988**
AB A new expression cassette comprises transcriptional regulatory, transcriptional initiation and translational initiation regions (which are functional in **Bacillus** sp. host), DNA encoding an **alkaline** protease, and transcriptional and translational termination regions. Preferably at least 1 marker gene and a temp.-sensitive bacterial origin of replication are present. The enzyme gene is preferably from **Bacillus** sp. strain **PB92** or has at least 90% homology with it. Also new are: (1) a transformed wild-type (or **mutant**), serine protease (non-) producing **Bacillus** sp. host, preferably containing at least 2 copies of the enzyme DNA sequence in its chromosome; (2) a method for producing serine protease in an **alkalophilic Bacillus** sp. host which comprises growing the transformed host in a culture medium under conditions which promote enzyme over-production; (3) a method for transforming **alkalophilic Bacillus** cells which comprises pretreating **Bacillus** cells with lysozyme (EC-3.2.1.17) at 20-37 deg to form protoplasts and introducing DNA in the presence of a fusogen; (4). . .
CT RECOMBINANT PROTEOLYTIC ENZYME, E.G. SERINE PROTEASE PREP., EXPRESSION CASSETTE CONSTRUCTION, DNA SEQUENCE, PLASMID PMAX-4 EXPRESSION IN **BACILLUS** SP. BACTERIUM

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     (FILE 'HOME' ENTERED AT 08:36:05 ON 28 MAR 2001)

     FILE 'REGISTRY' ENTERED AT 08:40:53 ON 28 MAR 2001
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     CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE,
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                 1   FILE NIOSHTIC
                 0*  FILE NTIS
                 0*  FILE OCEAN
                 0*  FILE PASCAL
                 0*  FILE PHIC
                 0*  FILE PHIN
              1315   FILE PROMT
                 0*  FILE SCISEARCH
                 0*  FILE USPATFULL
                 0*  FILE WPINDEX
L2              QUE L1
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FILE 'BIOSIS, AGRICOLA, BIOBUSINESS, PROMT, CIN, DRUGMONOG2, CEN, DRUGLAUNCH, EMBASE, NIOSHTIC' ENTERED AT 08:48:58 ON 28 MAR 2001

L3 60854 S L2
L4 2633 S L3 AND ALKAL?
L5 423 S L4 AND BACILLUS?
L6 9 S L5 AND CHROMOS?
L7 6 DUP REM L6 (3 DUPLICATES REMOVED)

=> d ibib ab 1

L7 ANSWER 1 OF 6 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 1
ACCESSION NUMBER: 1997:514347 BIOSIS
DOCUMENT NUMBER: PREV199799813550
TITLE: Sequencing of regions downstream of addA (98 degrees) and citG (289 degrees) in **Bacillus** subtilis.
AUTHOR(S): Medina, N.; Vannier, F.; Roche, B.; Autret, S.; Levine, A.;
Seror, S. J. (1)
CORPORATE SOURCE: (1) Inst. Genetique Microbiol., URA CNRS 2225, Univ. Paris XI, Batiment 409, 91405 Orsay Cedex France
SOURCE: Microbiology (Reading), (1997) Vol. 143, No. 10, pp. 3305-3308.
ISSN: 1350-0872.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The nucleotide sequence of 17-3 kbp downstream of addA (980) on the **Bacillus** subtilis **chromosome** was determined. Twenty putative ORFs were identified. Three of them coincided with known B. subtilis genes, addA, sbcD and wprA. The product of four other ORFs showed similarity to SbcC of Clostridium perfringens, CotH of B. subtilis, 2-hydroxyhepta-2,4-diene-1,7-diodate isomerase of Methanococcus jannaschi and a putative ORF of Pseudomonas syringae. In addition, a sequence of 7.6 kbp downstream of dtG (1890) was analysed. Among 10 putative ORFs identified, two coincided with known genes, dtG and mrgA, whilst three showed homology with X86780, a sensory protein kinase of Streptomyces hygroscopicus, an **alkaline** phosphatase regulatory protein and a hypothetical protease, YyxA, of B. subtilis.

=> d ibib ab 2

L7 ANSWER 2 OF 6 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 2
ACCESSION NUMBER: 1996:20475 BIOSIS
DOCUMENT NUMBER: PREV199698592610
TITLE: Extracellular enzyme synthesis in a sporulation-deficient strain of **Bacillus** licheniformis.
AUTHOR(S): Fleming, Alastair B.; Tangney, Martin; Jorgensen, Per L.; Diderichsen, Borge; Priest, Fergus G. (1)
CORPORATE SOURCE: (1) Dep. Biol. Sci., Heriot Watt Univ., Edinburgh EH14 4AS UK
SOURCE: Applied and Environmental Microbiology, (1995) Vol. 61, No. 11, pp. 3775-3780.
ISSN: 0099-2240.
DOCUMENT TYPE: Article
LANGUAGE: English
AB A deletion of the spoIIAC gene of **Bacillus** licheniformis was prepared in vitro by using the splicing-by-overlap-extension technique. This gene was introduced into B. licheniformis on a temperature-sensitive plasmid, and following integration and excision from the **chromosome**, a precisely located deletion on the **chromosomal** gene was prepared. The mutated bacterium was totally asporogenous and formed abortively disporic cells characterized by asymmetric septa at the poles of the cells. Qualitative plate tests revealed that the bacterium synthesized normal levels of DNase,

polygalacturonate lyase, protease, RNase, and xylanase, but the hydrolysis zones due to beta-1,3-glucanase and carboxymethyl cellulose activity were smaller in the mutant than in the parent strain. The synthesis of **alkaline** protease was the same in batch cultures of the mutant and the parent during prolonged incubation for 72 h, but the alpha-amylase yields were reduced by about 30% by the mutation.

=> d ibib ab 3

L7 ANSWER 3 OF 6 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1991:248679 BIOSIS
DOCUMENT NUMBER: BA91:129234
TITLE: CLONING CHARACTERIZATION AND MULTIPLE **CHROMOSOMAL** INTEGRATION OF A **BACILLUS ALKALINE** PROTEASE GENE.
AUTHOR(S): VAN DER LAAN J C; GERRITSE G; MULLENERS L J S M; VAN DER HOEK R A C; QUAX W J
CORPORATE SOURCE: ROYAL GIST-BROCADES N.V., RES. DEVELOPMENT, P.O. BOX 1, 2600 MA DELFT, THE NETHERLANDS.
SOURCE: APPL ENVIRON MICROBIOL, (1991) 57 (4), 901-909. CODEN: AEMIDF. ISSN: 0099-2240.
FILE SEGMENT: BA; OLD
LANGUAGE: English

AB Extracellular **Bacillus** proteases are used as additives in detergent powders. We identified a **Bacillus** strain that produces a protease with an extremely **alkaline** pH optimum; this protease is suitable for use in modern akaline detergent powders. The **alkalophilic** strain **Bacillus** alcalophilus PB92 gene encoding this high-**alkaline** serine protease was cloned and characterized. Sequence analysis revealed an open reading frame of 380 amino acids composed of a signal peptide (27 amino acids), a prosequence (84 amino acids), and a mature protein of 269 amino acids. Amino acid comparison with other serine proteases shows good homology with protease YaB, which is also produced by an alkatophilic **Bacillus** strain. Both show moderate homology with subtilisins but show some remarkable differences from subtilisins produced by neutrophilic bacilli. The prosequence of PB92 protease has no significant homology with prosequences of subtilisms. The abundance of negatively charged residues in the prosequences of PB92 protease is especially remarkable. The cloned gene was used to increase the production level of the protease. For this purpose the strategy of gene amplification in the original **alkalophilic Bacillus** strain was chosen. When introduced in a multicopy plasmid, the recombinant strain was unstable; under production conditions, plasmid segregation occurred. More stable ways of gene amplification were obtained by **chromosomal** integration. This was achieved by (i) homologous recombination, resulting in a strain with two tandemly arranged genes, and (ii) illegitimate recombination, resulting in a strain with a second copy of the protease gene on a locus not adjacent to the originally present gene. Both strains showed increased production and were more stable than the plasmid-containing strain. Absolute stability was only found when nontandem duplication occurred. This method of gene amplification circumvents stability problems often encountered in gene amplification in **Bacillus** species when plasmids or tandemly arranged gens in the **chromosome** are used.

=> d ibib ab 3

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ACCESSION NUMBER: 1991:248679 BIOSIS
DOCUMENT NUMBER: BA91:129234
TITLE: CLONING CHARACTERIZATION AND MULTIPLE **CHROMOSOMAL**

INTEGRATION OF A **BACILLUS ALKALINE** PROTEASE GENE.

AUTHOR(S): VAN DER LAAN J C; GERRITSE G; MULLENERS L J S M; VAN DER HOEK R A C; QUAX W J

CORPORATE SOURCE: ROYAL GIST-BROCADES N.V., RES. DEVELOPMENT, P.O. BOX 1, 2600 MA DELFT, THE NETHERLANDS.

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FILE SEGMENT: BA; OLD

LANGUAGE: English

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=> d ibib ab 4

L7 ANSWER 4 OF 6 BIOBUSINESS COPYRIGHT 2001 BIOSIS DUPLICATE 3

ACCESSION NUMBER: 91:40338 BIOBUSINESS

DOCUMENT NUMBER: 0362748

TITLE: Genetic manipulation of **Bacillus** amyloliquefaciens.

AUTHOR: VEHMAANPERA J; STEINBORN G; HOFEMEISTER J

CORPORATE SOURCE: RES. LAB. ALKO LTD., P.O. BOX 350, SF-00101 HELSINKI, 10, FINL.

SOURCE: JOURNAL OF BIOTECHNOLOGY, (1991) VOL.19, NO.2-3, P.221-240.

FILE SEGMENT: NONUNIQUE

LANGUAGE: ENGLISH

AB Application of modern gene technology to strain improvement of the industrially important bacterium **Bacillus** amyloliquefaciens is reported. Several different plasmid constructions carrying the .alpha.-amylase gene (amyE) from B. amyloliquefaciens were amplified in this species either extrachromosomally or intrachromosomally. The amyE gene cloned on a pUB110-derived high copy plasmid pKTH10 directed the

highest yields both in rich laboratory medium and in crude industrial medium. The .alpha.-amylase activity, when compared with the parental strain, was enhanced up to 20-fold in the pKTH 10 transformant. This strain showed decreased activities for other exoenzymes, such as proteases and .beta.-glucanase suggesting common limiting resources in the processing of these enzymes. Deletions were made in vitro in genes encoding neutral (nprE), **alkaline** (aprE) protease and .beta.-glucanase (bglA). The engineered genes were cloned into the thermosensitive plasmid pE194, and the resulting plasmids were used to replace the corresponding wild type **chromosomal** genes in B. amyloliquefaciens by integration-excision at non-permissive temperature. The double mutant deficient in the major proteases (.DELTA.nprE.DELTA.aprE) showed about a 2-fold further enhancement in .alpha.-amylase production in the industrial medium compared with the relevant wild type background, both when plasmid-free and when transformed with pKTH10; this strain also produced elevated levels of the **chromosomally**-encoded .beta.-glucanase; pKTH10 was stably maintained both in the wild type strain and in the .DELTA.nprE.DELTA.aprE mutant. We suggest that the higher yields in .alpha.-amylase and .beta.-glucanase in the .DELTA.nprE.DELTA.aprE strain are primarily due to improved access to limiting resources, and that decreased proteolytic degradation may have had a secondary role in retaining the high activity obtained.

=> d ibib ab 5

L7 ANSWER 5 OF 6 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1987:129755 BIOSIS
DOCUMENT NUMBER: BA83:68816
TITLE: CHARACTERIZATION AND MAPPING OF THE **BACILLUS**-SUBTILIS PRT-R GENE.
AUTHOR(S): YANG M; SHIMOTSU H; FERRARI E; HENNER D J
CORPORATE SOURCE: DEP. CELL GENET., GENENTECH INC., SOUTH SAN FRANCISCO, CALIF. 94080, USA.
SOURCE: J BACTERIOL, (1987) 169 (1), 434-437.
CODEN: JOBAAY. ISSN: 0021-9193.
FILE SEGMENT: BA; OLD
LANGUAGE: English

AB A gene from **Bacillus** natto encoding a 60-amino-acid peptide has been previously described that, when cloned on a high-copy plasmid in B. subtilis, enhances production of **alkaline** protease, neutral protease, and levansucrase. An identical gene was isolated from B. subtilis and caused a similar phenotype when placed on a high-copy plasmid. Genetic mapping localized this gene near metB, distant from other pleiotropic genes causing similar effects. Deletion of this gene from the B. subtilis **chromosome** had no obvious phenotypic effect.

=> d ibib ab 6

L7 ANSWER 6 OF 6 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1979:264656 BIOSIS
DOCUMENT NUMBER: BA68:67160
TITLE: THE NATURE OF MUTATIONS DETERMINING THE ABILITY OF **BACILLUS**-SUBTILIS A-50 TO SPORULATE AT HIGH CONCENTRATIONS OF GLUCOSE IN THE MEDIUM.
AUTHOR(S): DOBRZHANSKAYA E O; EROKHINA L I; BOL'SHAKOVA T N
CORPORATE SOURCE: ALL-UNION RES. INST. GENET. SEL. IND. MICROORG., MOSCOW, USSR.
SOURCE: GENETIKA, (1978) 14 (7), 1175-1184.
CODEN: GNKAA5. ISSN: 0016-6758.

FILE SEGMENT: BA; OLD
LANGUAGE: Russian

AB The ability of B. subtilis A-50 to sporulate in medium containing high glucose concentrations is caused by at least 2 mutation types: pts mutations and cat (or tgl) mutations, both of them affecting differently the level of **alkaline** protease synthesis. The decrease of the level of enzyme activity in the case of pts mutation (gluR3 mutant) occurs at the expense of glucose transport disturbance. The mutation cat (tgl) (mutant gluR5) causes an increase in enzyme synthesis at the expense of catabolic resistance to glucose of genes controlling **alkaline** protease synthesis and spore formation in B. subtilis A-50. cat5(gluR5) and pts3(glu)R3) mutations are located on the **chromosome** of B. subtilis in the metD and argC regions, respectively. The over-synthesis of **alkaline** protease characteristic of B. subtilis A-50 is controlled by the polygenic system, as the level of **alkaline** protease synthesis in argA+ transformants makes up 25% of the level of activity of the original strain. The productivity of B. subtilis A-50 can be enhanced by introducing an additional cat mutation.